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Effect of the alien invasive bivalve *Corbicula fluminea* on the nutrient dynamics under climate change scenarios

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ABSTRACT

The main aim of this study was to evaluate the impact of the alien invasive bivalve *Corbicula fluminea* (Müller, 1774) in the nutrient dynamics of temperate estuarine systems (oligohaline areas) under climate change scenarios.

The scenarios simulated shifts in climatic conditions, following salinity (0 or 5) and temperature (24 or 30 °C) changes, usual during drought and heat wave events. The effect of the individual size/age (different size classes with fixed biomass) and density (various densities of <1 cm clams) on the bioturbation-associated nutrient dynamics were also evaluated under an 18-day laboratory experimental setup.

Results highlight the significant effect of *C. fluminea* on the ecosystem nutrient dynamics, enhancing the efflux of both phosphate and dissolved inorganic nitrogen (DIN) from the sediments to the water column. Both drought and heat wave events will have an impact on the DIN dynamics within *C. fluminea* colonized systems, favouring a higher NH_4-N efflux.

The population structure of *C. fluminea* will have a decisive role on the impact of the species, with stronger nutrient effluxes associated with a predominantly juvenile population structure.

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1. Introduction

Invasive alien species (IAS) introduction has increased considerably in aquatic ecosystems in recent decades, as a result of expanding commerce, aquaculture and shipping activities (Sousa et al., 2008b; Chan and Bendell, 2013; Simberloff et al., 2013). Traits such as short life span, early maturity, rapid growth and high fecundity contribute to the success of invasive species, by promoting the development of dense invader populations (McMahon, 2002; Crespo et al., 2015). These, in turn, outperform native populations in rapidly repopulating colonized systems, after mortality events in unstable ecosystems. In particular, ecosystem engineers and filter-feeding IAS have been reported to strongly impact biodiversity and ecosystem functioning (Chan and Bendell, 2013).

Corbicula fluminea O.F. Müller [1774] is one of the most successful invasive species worldwide, and is present in several European estuarine and freshwater areas (Franco et al., 2012; McMahon, 2002; Sousa et al., 2008b; Crespo et al., 2015). It can represent as much as 90% of the macrobenthic community production (Sousa et al., 2008a) with abundance and biomass of more than 4000 ind. m^{-2} and 550 g AFDW m^{-2}, respectively (Sousa et al., 2008b). Reported *C. fluminea* ecosystem impacts span from changes in sediment characteristics, via bioturbation and shell production (Sousa et al., 2009), to phytoplankton population control and turbidity decreases due to high filtration rates (Phelps, 1994; Sousa et al., 2008; Majdi et al., 2014), or the disruption of food chains as a result of its high productivity and massive die-offs (Sousa et al., 2008b, 2012). Additional impacts of *C. fluminea* encompass

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alterations in the biogeochemical cycles, through a myriad of mechanisms. For instance, pedal feeding promotes alterations in the abiotic characteristics of the top layer of the sediments, resulting in high rates of nitrogen excretion (Vaughn and Hakenkamp, 2001). In a different study, the presence of bivalves (including *C. fluminea*) in the Chesapeake Bay system was modelled to remove 14%–40% of the carbon load, 11%–23% of the nitrogen load, and 37%–84% of the phosphorus load, by reducing net phytoplankton primary production by 31%–44% (Cerco and Noel, 2010).

A distinct mechanism which may also affect biogeochemical dynamics is bioturbation and bioirrigation; the first influences the distribution of sediment particles (by sediment reworking), while the latter will have an impact on solutes (by burrow ventilation) within the sediment and at the sediment–water interface (Majdi et al., 2014). These interactions result in changes in sediment characteristics, such as grain size, organic matter content, sediment stability and aggregation, porosity and water retention capacity, which will affect interstitial water characteristics, oxygen and nutrient concentrations and release to the water column and redox potential, among other abiotic factors that control biogeochemical processes (Sousa et al., 2009).

Global climate change, with altered frequencies and intensities of extreme weather events (floods, droughts and heat waves), together with mean sea level rise, are expected to have adverse effects on natural and human systems (IPCC, 2014). The effects of this climatic and environmental instability on biological communities are unpredictable, as are subsequent outcomes to the ecosystem functioning (Wrona et al., 2006). Such effects are especially important for invasive species, because they are often not yet at equilibrium, represent a significant component of aquatic communities and can interact strongly with native species (McDowell et al., 2014). Alterations of environmental conditions due to sudden, dramatic events associated with climate change may also open opportunities for the proliferation of invasive species, previously unable to sustain viable populations due to physical environmental constraints (Montoya and Raffaelli, 2010; Crespo et al., 2015, 2017).

Massive mortality events have been reported for *C. fluminea* (Oliveira et al., 2015; Sousa et al., 2012) associated with extreme events such as droughts, heat waves, decrease of oxygen concentration in the water, increase of pollution and unmet metabolic demand (Petter et al., 2014; Oliveira et al., 2015), particularly due to their combined impacts (e.g. Crespo et al., 2017). However, the species may also strive under some temperature increase scenarios (Rosa et al., 2012) and is known to tolerate some salinity (Crespo et al., 2017). In fact, temperature and salinity have been found to affect rates of oxygen consumption, ammonium excretion, CO_2 emission and mortality (Xiao et al., 2014; Crespo et al., 2017). Yet, little information is available on the possible consequences of climate change on the effect of this IAS for ecosystems functions, such as nutrient release from sediment, its dynamics and changes in the sediment biogeochemistry. Therefore, the main objective of this research was to evaluate the effect of *Corbicula fluminea* in the nutrient fluxes to the water column under extreme climatic events (rise in temperature and rise in salinity following a drought event), and considering distinct population structure (size) and density. Overall, it was expected to gain information on the impact of this species under such climate scenarios, which will predictably increase, to aid for future ecosystem conservation and management purposes.

2. Methods

2.1. Faunal and sediment collection

Corbicula fluminea individuals were collected in the well-described Mondego Estuary (Grilo et al., 2011; Dolbeth et al., 2011; Franco et al., 2012). Samples were collected in the oligohaline upper reaches (40° 9′47.91″N, 8°40′12.42″W), still under tidal influence despite the considerable distance to the river mouth. Conditions in the collection site included sandy sediments and low macrofauna diversity which was dominated by *C. fluminea* (Crespo et al., 2017). Specimens were collected by hand and transported refrigerated to the laboratory, in water collected *in situ*. Sediment for the experimental tanks was collected in the same site. Fauna were acclimated to laboratory conditions for at least 48h prior to the start of experiments (Magni et al., 2000; Hakenkamp and Palmer, 1999; Atkinson et al., 2011).

2.2. Experimental tank' assemblage and experimental design

The experimental apparatus (Fig. 1) consisted of glass tanks with $12 \times 12 \times 35$ cm (internal dimensions). The tanks were assembled with 10 cm of the collected sediment and filled with 20 cm of water ($\approx$3 L). Sediment was thoroughly homogenized to assure comparable conditions across tanks, and macrofauna was previously removed by CO_2 enriched water treatments. Two different salinity treatments were selected, in accordance with the usual salinity range measured in the oligohaline estuarine areas (freshwater 0, and 5, synthetic salt water prepared with ultrapure water and Tropic Marin® SEA SALT synthetic sea salt mix), and two water temperature treatments ($\approx$24 °C, the reported summer water temperature at the collection site (Modesto et al., 2013), and 30 °C, representing field water temperature in extreme conditions, such as during heat waves occurrence (Mouthon and Daufresne, 2006; Grilo et al., 2011)).

Water in each tank was replaced after 24 h to remove excess nutrients associated with sediment manipulation during assembly. Different *Corbicula fluminea* size classes (representing different cohorts) were tested, according to its population structure: small size individuals (<1 cm, ~1 year old), medium size (2–2.5 cm, ~2 years old) and large size (>3.5 cm, >3 years). For each treatment, individuals of each size class were stocked at the same total biomass to represent the densities ($\approx$18.8 g wet weight per 144 cm^{-2} tank area) and the population structure found in the field during summer conditions (Crespo et al., 2017). This corresponded to different density levels: 13 small-sized *C. fluminea* individuals; 2 medium-sized *C. fluminea* individuals; 1 large-sized *C. fluminea* individual (Fig. 1). Each treatment was performed in triplicate. Organisms were not fed during the experiments, but were assumed to feed on the sediment organic matter content (sediment OM = 0.4 ± 0.2%).

For the small-sized individuals alone, the most abundant size class at the collection site, the density effect was also tested, using a control with no fauna and three density treatments with 6, 12 and 24 individuals (mean biomass per tank: 9.0, 18.8 and 35.7 g wet weight per 144 cm^{-2} tank area respectively). For this density experiment, tanks were filled with 3 cm of sand and freshwater (8.6 L) at $\approx$ 24 °C. Each density treatment was done in triplicate.

For comparison purposes, in all experiments Control tanks (without *C. fluminea*) were run with the same experimental conditions as those with bivalves (both salinity and temperature treatments). Tanks were permanently aerated and exposed to natural light conditions ($\approx$10h of light with variable intensity, depending on weather conditions). The experiment was held in a constant-temperature room ($\approx$24 °C) and the higher tested temperature ($\approx$30 °C) was achieved using aquarium heaters (Eheim Jäger 3612 Aquarium Heater). Bivalve mortality was negligible, and was only observed in the first two days of the experiment (assumed to be related with acclimation to the experimental salinity and temperature conditions). When it occurred, the dead bivalve was

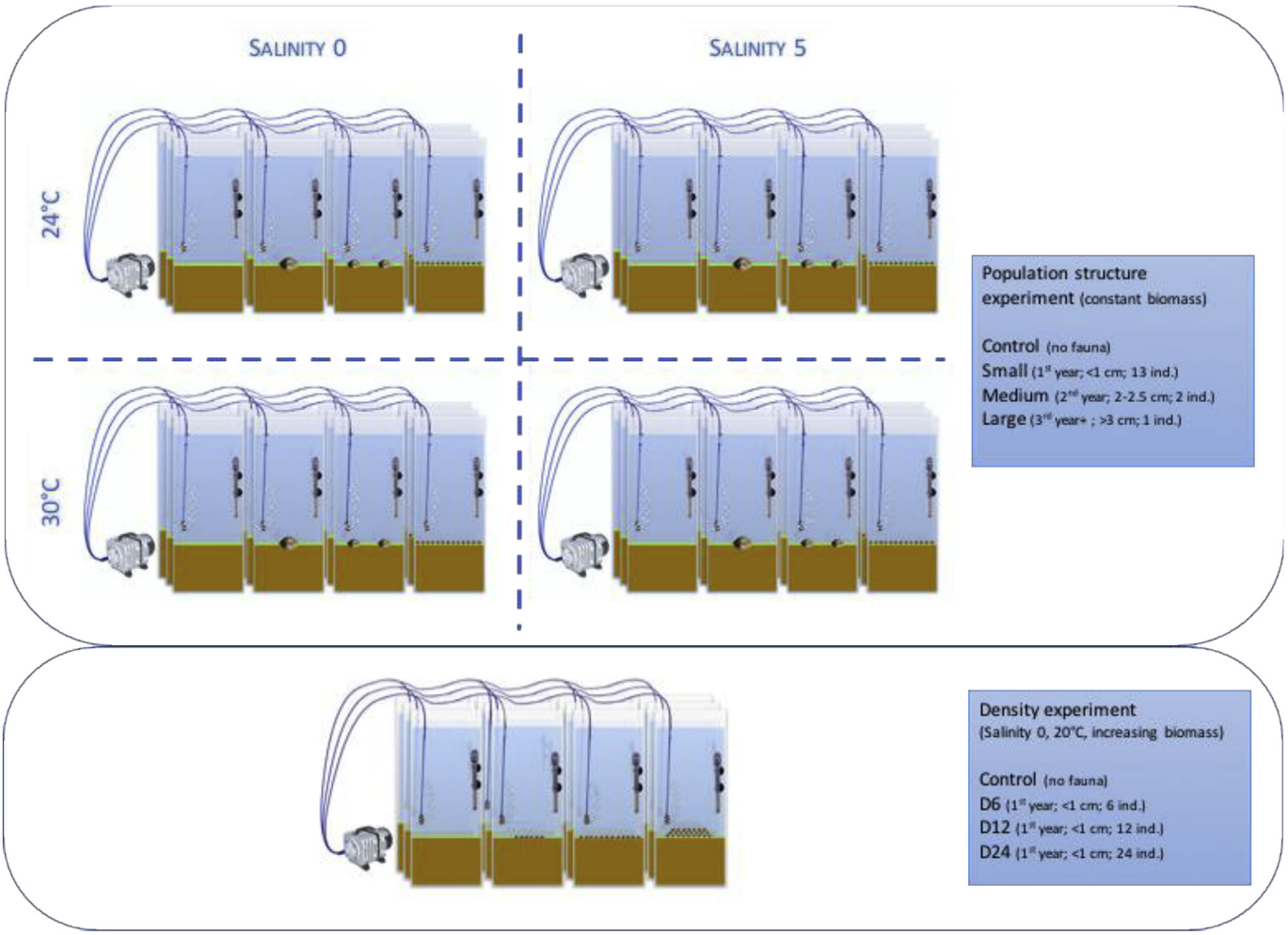

Fig. 1. Experimental design schematics.

replaced with a similar sized individual to maintain biota biomass.

For both experiments, salinity, temperature, pH and dissolved oxygen concentration were monitored in the water column and water samples (10 mL) collected at day 0, day 3, day 6, day 12 and day 18 of incubation from each tank, for nutrient analysis. The concentration of dissolved inorganic nutrients (PO_4-P, NOx-N and NH_4-N) in the water column was analysed with Continuous Flow Analyzer Skalar Sanplus with Segmented Flow Analysis (SFA), using the Skalar methods: M461-318 (EPA 353.2), M155-008R (EPA 350.1) and M503-555R (Standard Method 450-P I). Changes in the concentrations of dissolved inorganic nutrients were monitored as a proxy for the processes occurring at the sediment water interface due, or not (control), to the presence of the tested population structure of bivalves. In the case of phosphorus, any changes in concentration of the inorganic dissolved form will result from mineralization processes (Zhang et al., 2011) or bivalve metabolism (Vaughn and Hakenkamp, 2001), whilst for nitrogen changes in concentration of the inorganic dissolved forms may involve biogeochemical processes taking place at the sediment water interface (e.g. coupled nitrification and denitrification) (Turek and Hoellein, 2015) or excretion of ammonium by living organisms (Liu et al., 2007; Zhang et al., 2011). Nevertheless, the control conditions will account for all these processes taking place at the sediment compartment without the influence of bioturbation and bioirrigation by macrofauna (Turek and Hoellein, 2015; Zhang et al., 2011).

2.3. Data analyses

The data were statistically tested with a Permutational Multivariate Analyses of Variance – PERMANOVA for a three-factor, crossed repeated measures design, to test the response of the concentration of nutrients to the fixed factors size (four levels: control, small, medium and large), salinity (two levels: 0 and 5) and temperature (two levels: 24 and 30), and with time as a repeated measure (3 replicates per time). PERMANOVA is an analysis of variance to test one or more factors, using permutation methods and on the basis of a resemblance matrix (Anderson et al., 2008). The only assumption is the exchangeability of samples and therefore it can be used for a repeated measures design (Anderson et al., 2008). Still, as the analysis was done for each nutrient alone, we treated each time point as separate variable and then performed a multivariate analysis among treatments, using the Euclidean distance as resemblance matrix (Anderson et al., 2008).

Prior to the PERMANOVA, the homogeneity of within-group dispersion among tested factors (size, salinity and temperature) was tested with PERMDISP analyses, also with the Euclidean distance as resemblance matrix, as PERMANOVA is sensitive to differences in the multivariate dispersion among groups. For the density experiment, both PERMDISP and PERMANOVA were also applied using the same procedure as with the size experiment (each data point as variable) on a Euclidean Distance basis to test the response of the nutrient content to the fixed factor density, with

four levels (control, 6 inds, 12 inds and 24 inds), with replication (3 replicates). All the analyses were performed using PRIMER v6 software with the PERMANOVA add-on package (Anderson et al., 2008).

Nutrient fluxes to the water column were expressed in terms of concentration change over time. The data obtained per unit of time was modelled by nonlinear regression analysis, using GraphPad Prism 6 (trial version) that uses the least-squares fitting method and the method of Marquardt and Levenberg for adjusting the variables; this method blends the method of linear descent and the method of Gauss-Newton. The one-phase exponential association model (eq. (1)) was used to fit data from water column nutrient concentrations:

$$C_t = C_0 + (C_{ss} - C_0)^*(1 - \exp(-k_m t)) \quad (1)$$

where C_t and C_{ss} are the concentrations at time t (d) and at steady-state, respectively; k_m is the mineralization rate constant (d^{-1}); C_0 is the concentration at time 0. To assess the goodness of the fit of the experimental data, the coefficient of determination (R^2) and the standard deviation of residues ($S_{x/y}$) were determined. A relatively high R^2 and low value of $S_{x/y}$ were used as criteria for good fit. For each case, the fitting was tested using three replicate concentrations at each studied time.

For each nutrient, a half-life (the time it takes to reach half of the equilibrium value) was calculated ($T_{b1/2}$) from the corresponding mineralization (k_m) rate constant, according to the relation $T_{b1/2} = \ln 2/k_m$.

3. Results

3.1. Experimental conditions

Measured salinity for the category "salinity 5" was 4.5 ± 0.3 (mean ± standard deviation). Regarding temperature, measured values for treatment 24 °C were 23.6 ± 1.0 °C, and for the 30 °C one 29.8 ± 1.2 °C. Dissolved oxygen concentrations were generally higher for the 24 °C treatment (8.0 ± 0.4 mg L^{-1}; 95%) than for the 30 °C (6.4 ± 0.6 mg L^{-1}; 85% ± 5), while pH varied within similar values for all treatments (7.1 ± 0.3).

Nutrient concentrations at time 0 (C0) were respectively 0.08 mg L^{-1} PO_4-P, 0.06 mg L^{-1} NO_3-N, 0.04 mg L^{-1} NH_4-N and 0.004 mg L^{-1} NO_2-N. At the last day of the experiment, PO_4-P concentrations ranged between 0.06 and 0.89 mg L^{-1}; NO_3-N concentrations ranged between 0.08 and 0.76 mg L^{-1}; NH_4-N concentrations ranged between 0.01 and 0.12 mg L^{-1}; the average NO_2-N concentration was 0.04 mg L^{-1}. However, nutrients showed distinct dynamics and patterns that will be discussed in the following section.

3.2. Population structure/environmental data experiment

Within-group dispersion was significant for the factor size for both PO_4-P (F = 8.59, p-perm = 0.001) and NH_4-N (F = 24.78, p-perm = 0.001). For NH_4-N alone, within group dispersion was also observed for salinity (F = 11.06, p-perm = 0.03).

The 2-way interaction size x temperature was significant for all nutrients, which means that size may have constrained the response to temperature (Table 1). In addition, for PO_4-P and NH_4-N, size x salinity was also significant (Table 1). Time was not included as an independent factor, however, differences with time were visible, with an asymptotic variation pattern for PO_4-P (Fig. 2A) and increasing pattern for NO_3-N through the study period (Fig. 2B), and also an increasing pattern for NH_4-N up to day 6 (Fig. 2C). Regarding the other factors, size was the variable with the highest variation component for all nutrients (not shown here), with highest nutrient release generally observed for the small and medium size *Corbicula* treatments.

For PO_4-P, significant differences were observed between all size classes' combinations for both salinities, with some exceptions for temperature (Table 1). Generally, PO_4-P release was highest for the small size classes at salinity 0, and for the medium class at salinity 5 and 30 °C (Fig. 2A). As mentioned, PO_4-P release rates were relatively rapid in the first days of the experiment (up to day 6) and progressively diminished until approaching an apparent equilibrium. Dissolved NO_3-N concentrations, in turn, continuously increased until day 12 and only started levelling off between days 12 and 18, with less pronounced differences between treatments (Fig. 2B). There were differences between the control and the other size classes, except for the large size individuals at 24 °C (Fig. 2B, Table 1).

While salinity was not significant, NO_3-N release was slightly lower for the control at 30 °C and slightly higher for the medium size classes (Table 1, Fig. 2B). Regarding NH_4-N, differences were observed between treatments until day 6 (highest for the medium sized *Corbicula*, with a maximum concentration of 2.0 mg L^{-1}), followed by a sharp decrease towards day 12 (Fig. 2C). Consequently, at the end of the experiment no differences were observed

Table 1
Summary of significant terms from the PERMANOVA analyses, using nutrient concentrations as dependent variables and size class (control, small, medium and large), salinity (0 and 5) and temperature (24 °C and 30 °C) as explanatory variables, with indication of the significant pairwise comparisons. Tested terms were: single ones - size, salinity, temperature, 2-way interactions - Size x Salinity, Size x Temperature, Salinity x Temperature, and the 3-way interaction Size x Salinity x Temperature.

Dependent variable	Significant terms	d.f.	Pseudo-F	p-value	Terms/levels of factor	P (perm)
PO_4-P	Size x Salinity	3	6.4878	0.001	- Salinity 0 and 5: all size combinations	<0.03
					- Control, Small and Large: Salinity 0 vs. Salinity 5	<0.01
	Size x Temperature	3	3.4159	0.015	- 24 °C: all size combinations, except Control vs. Medium	<0.01
					- 30 °C: all size combinations, except Small vs. Medium	<0.005
					- Large size: 24 °C vs. 30 °C	<0.05
NO_3-N	Size x Temperature	3	2.7054	0.039	- 24 °C: all size combinations, except Control vs. Large	<0.02
					- 30 °C: all size combinations, except Small vs. Medium	<0.01
					- Control: 24 °C vs. 30 °C	0.001
					- Small size: 24 °C vs. 30 °C	0.018
NH_4-N	Size x Salinity	3	5.1773	0.001	- Salinity 0: all size combinations, except Small vs Medium	<0.02
					- Salinity 5: all size combinations	<0.03
					- All sizes: Salinity 0 vs. Salinity 5	<0.03
	Size x Temperature	3	4.4135	0.007	- 24 °C: all size combinations, except Control vs. Large and Small vs. Medium	<0.002
					- 30 °C: all size combinations	<0.01
					- Medium size: 24 °C vs. 30 °C	0.021

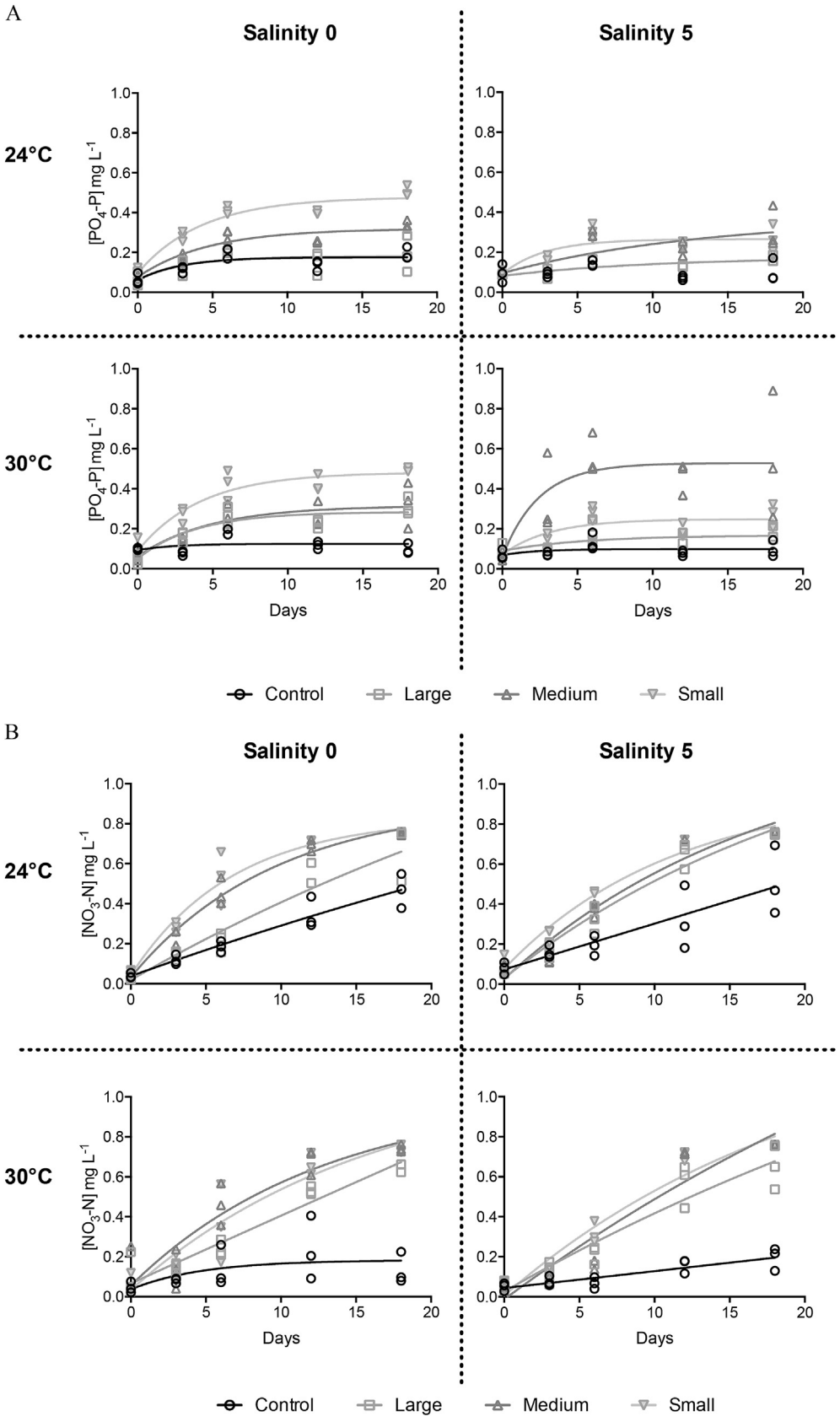

Fig. 2. The concentration of dissolved inorganic nutrients in the water column: over time for each size class, per salinity and temperature. A fitted regression line was added to plot to clarify the variation patterns. A) PO_4-P; B) NO_3-N; and C) NH_4-N.

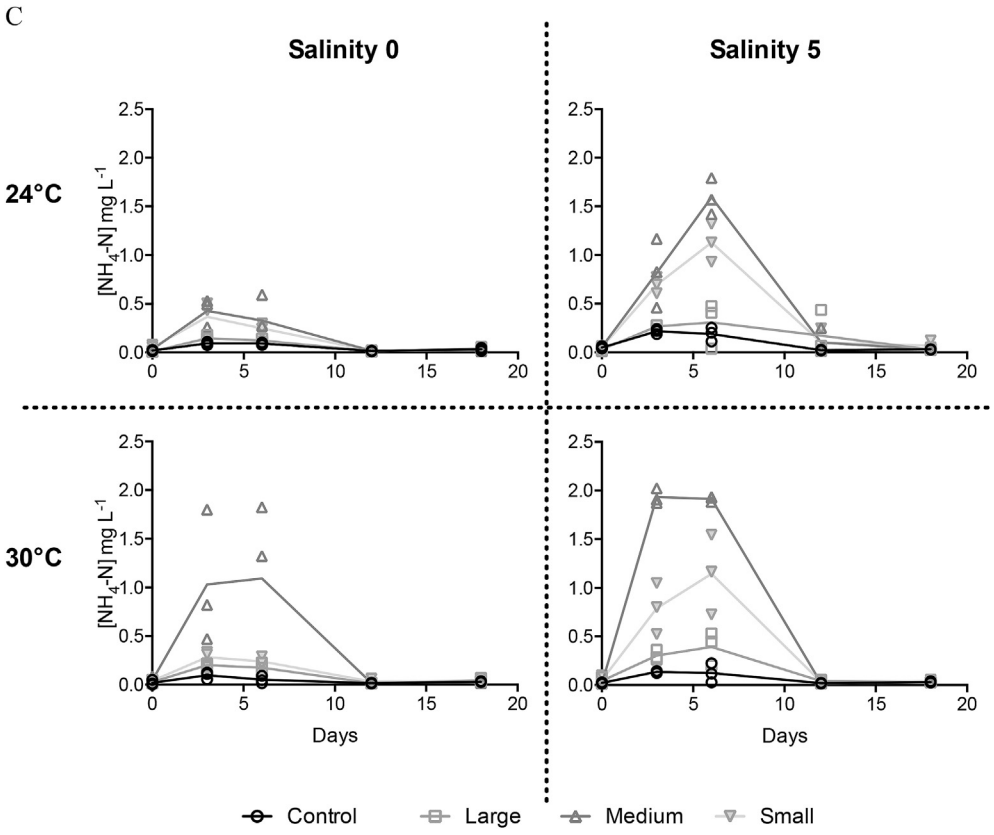

Fig. 2. (*continued*).

between treatments. Nitrate accounted for most dissolved inorganic nitrogen (DIN) in all treatments (89% median value). Considering that time was not accounted as an independent factor, significant differences were detected between control and all size combinations for both salinity and temperatures tested, except for the larger individuals at salinity 0 (Table 1, Fig. 2C). Despite the dispersion in size and salinity, ammonium release was, again, generally highest for the medium and small size class treatments, particularly at the higher salinity and higher temperature (Fig. 2C). Nitrite concentrations did not show a specific pattern, and overall remained low throughout the experiment.

A more in-depth study on the kinetics of the nutrient release was performed for phosphorus and nitrate, the prevalent form of nitrogen (Fig. 2A and B, Table 2). Generally, a pseudo-first order kinetics model explained nutrient release efficiently, with some exceptions due to the dispersion between replicates or the absence of nutrient release (mainly in Control Tanks). Results for PO_4-P confirm the equilibrium at the end of the experimental period, with predicted C_{ss} similar to measured concentrations, and $T_{b1/2}$ in the range of 2–9 days (Table 2). For NO_3-N, however, modelling suggests that equilibrium was not attained at the end the 18-day experimental period, with predicted half-life periods of up to 30 days and steady-state concentrations higher than those observed at day 18 (Table 2). Additionally, while no significant differences were observed between most treatments at day 18, kinetic modelling results suggest that if allowed to reach equilibrium, NO_3-N concentrations would differ between treatments.

3.3. Density experiment

Within-group dispersion was non-significant in the density experiment. Density was significant for all nutrients (p-perm <0.02). However, the pairwise comparisons were not significant (p-perm>0.08) due to the low number of possible unique permutations (10). Still, some patterns were apparent, with the concentrations of the control generally lower than the ones with fauna (Fig. 3). Phosphate mineralization was faster in the initial 6 days of the experiment, and tended towards equilibrium at the end of the experimental period (Fig. 3A). Density of individuals influenced phosphate release, with the highest concentrations (up to 0.9 mg L^{-1}) recorded in the D24 tanks. Inversely, no clear pattern was observed between density treatments for nitrate (Fig. 3B) or ammonium (Fig. 3C). Generally, nutrient concentrations were within range of the ones observed in the size experiment at equivalent salinity and temperature conditions (i.e. temperature 24 °C and salinity 0, Fig. 3).

Kinetic modelling revealed that, similarly to what was observed for the size experiment, *Corbicula* density had distinctive impacts in the nutrient dynamics (Table 3). While the phosphate concentration was positively correlated with bivalve density, with increasing C_{ss} concentrations and half-life times from control to D24 tanks, nitrate concentrations at the end of the experiment were comparable in all treatments. However, the mineralization rates of D12 and D24 treatments were considerably faster, reaching equilibrium before day 3, while D6 and control tanks $T_{b1/2}$ were, respectively, 5 and 9 days (Table 3).

4. Discussion

4.1. The effect of C. fluminea on nutrient dynamics

Considerable scientific attention has been devoted to the impact of *Corbicula fluminea* colonization on invaded ecosystems, in light of its successful geographical expansion and ecological effects (e.g.

Table 2
Estimated kinetic parameters of the nutrient (PO_4-P and NO_3-N) release from the size-class experiment tanks (n = 3 for each time point). C0, concentration at time 0; Css, concentration at steady state; km: mineralization rate constant (d^{-1}); $T_{b1/2}$: half-life (d); R_2: determination coefficient; SE: standard error.

				C_0 (mg L^{-1}) ± SE	C_{SS} (mg L^{-1}) ± SE	K_m (d^{-1}) ± SE	$T_{b1/2}$	R^2	$S_{x/y}$
PO_4-P	24 °C	Sal 0	Control	0.061 ± 0.022	0.18 ± 0.02	0.35 ± 0.21	1.98	0.60	0.039
			Large	0.061 ± 0.032	0.18 ± 0.03	0.31 ± 0.25	2.25	0.44	0.056
			Medium	0.076 ± 0.022	0.32 ± 0.02	0.21 ± 0.06	3.24	0.87	0.038
			Small	0.096 ± 0.024	0.48 ± 0.02	0.23 ± 0.05	2.98	0.93	0.041
		Sal 5	Control	–	–	–	–	–	–
			Large	0.082 ± 0.016	0.18 ± 0.05	0.10 ± 0.12	6.92	0.57	0.028
			Medium	0.095 ± 0.040	0.37 ± 0.23	0.07 ± 0.11	9.23	0.57	0.074
			Small	0.091 ± 0.030	0.27 ± 0.02	0.35 ± 0.02	1.96	0.67	0.051
	30 °C	Sal 0	Control	0.093 ± 0.025	0.13 ± 0.02	0.41 ± 1.06	1.69	0.08	0.044
			Large	0.050 ± 0.026	0.28 ± 0.02	0.26 ± 0.09	2.67	0.82	0.045
			Medium	0.061 ± 0.040	0.32 ± 0.04	0.21 ± 0.13	3.38	0.71	0.069
			Small	0.089 ± 0.031	0.48 ± 0.03	0.24 ± 0.06	2.92	0.90	0.054
		Sal 5	Control	0.068 ± 0.020	0.099 ± 0.014	0.46 ± 1.02	1.50	0.12	0.035
			Large	0.090 ± 0.026	0.17 ± 0.03	0.18 ± 0.23	3.79	0.30	0.047
			Medium	0.059 ± 0.099	0.53 ± 0.07	0.41 ± 0.28	1.69	0.57	0.17
			Small	0.071 ± 0.033	0.25 ± 0.03	0.31 ± 0.18	2.21	0.62	0.057
NO_3-N	24 °C	Sal 0	Control	0.038 ± 0.026	2.0 ± 4.2	0.014 ± 0.033	50.4	0.92	0.050
			Large	0.018 ± 0.041	1.9 ± 2.7	0.022 ± 0.038	30.8	0.91	0.078
			Medium	0.031 ± 0.027	0.91 ± 0.08	0.10 ± 0.02	6.66	0.97	0.050
			Small	0.042 ± 0.035	0.83 ± 0.06	0.15 ± 0.03	4.70	0.96	0.063
		Sal 5	Control	–	–	–	–	–	–
			Large	0.030 ± 0.031	1.4 ± 0.5	0.044 ± 0.023	15.7	0.96	0.058
			Medium	0.026 ± 0.042	1.2 ± 0.4	0.057 ± 0.030	12.2	0.94	0.079
			Small	0.076 ± 0.027	0.97 ± 0.11	0.089 ± 0.021	7.83	0.97	0.049
	30 °C	Sal 0	Control	0.036 ± 0.053	0.18 ± 0.06	0.21 ± 0.27	3.27	0.28	0.094
			Large	–	–	–	–	–	–
			Medium	0.055 ± 0.060	1.0 ± 0.3	0.078 ± 0.046	8.90	0.87	0.11
			Small	0.033 ± 0.052	1.1 ± 0.4	0.062 ± 0.039	11.1	0.90	0.096
		Sal 5	Control	–	–	–	–	–	–
			Large	0.026 ± 0.047	2.1 ± 3.4	0.021 ± 0.039	33.8	0.89	0.090
			Medium	0.000 ± 0.057	2.5 ± 3.6	0.023 ± 0.040	30.7	0.91	0.11
			Small	0.005 ± 0.041	1.4 ± 0.6	0.048 ± 0.028	14.6	0.94	0.077

Crespo et al., 2015). Considering the high population density and productivity of the species, significant biogeochemical effects are expected in the sediment/water column interface, and have been reported by other authors (Majdi et al., 2014; Chen et al., 2016; Zhang et al., 2011, 2013).

Overall, our study reinforces the significant effect of *Corbicula fluminea* on the nutrient dynamics and release into the water column. Despite some within-treatment dispersion, which can be associated with the low number of replicates per treatment, at an ecologically relevant biomass per m^2, all size classes, salinity and temperature treatments differed, to some extent, from the Control treatment with no fauna. While no sediment or pore-water nutrient concentrations were quantified, differences from control treatments derive from bivalve impact on sediment geochemistry and/or metabolism, in a mass-balance effect between sediment and dissolved nutrient pools, which tends to reach equilibrium. The influence exerted by *C. fluminea* on the nutrient release and dynamics probably results from a combination of mechanisms, comprising both metabolic and physical effects.

Still, for the same biomass, the population structure emerges as the main factor governing nutrient release, with enhanced dissolved concentrations in the small and medium sized *Corbicula*' tanks. These enhanced nutrient fluxes associated with smaller-sized clams may result either from their increased sediment reworking activities, when compared with larger individuals, as reported by Majdi et al. (2014) and Crespo et al. (2018), or by the increased density of individuals in the small size treatments, which occupy a larger surface area. While large clams may induce displacement of particles deeper into the sediment, small clams showed the highest net sediment reworking activity, which should increase the oxic–anoxic sediment interface, and therefore promote nutrient release (e.g. Mortimer et al., 1999; Gilbert et al., 1998). Bioturbation quantification was performed in this experiment and results seem to support this hypothesis, with much higher sediment reworking rates in medium and small sized *C. fluminea*' tanks, when compared with large sized *C. fluminea* ones (Fig A1, Crespo et al., 2018). Additionally, smaller clams have been reported to be metabolically more active, exhibiting higher oxygen consumption rates, ammonia excretion rates and CO_2 emission rates than large *C. fluminea* individuals (Xiao et al., 2014; Mortimer et al., 1999). This higher metabolic rate (and higher number of individuals) would favor an increase in the net nutrient release through excretion, which might have substantial effect on river nutrient cycling, especially in summer (Lauritsen and Mozley, 1989).

The observed enhancement of phosphorus release by the presence of *C. fluminea* has previously been reported, and attributed to the increased diffusion, enhanced advection between the pore water and overlying water and bivalve metabolism (Zhang et al., 2011). For transitional waters, the increase in the oxic–anoxic sediment interface and the enhancement of sediment total microbial activity may accelerate organic matter degradation, and thus release additional phosphorus to the water column (Lillebø et al., 1999). In a different study, Chen et al. (2016) observed up to 157% increase in P fluxes from *C. fluminea* bioturbated sediments, associated to the effects of bivalve respiration in the sediment. In addition, an inverse relationship between unionid bivalve size and phosphorus excretion rates has been reported (Vaughn and

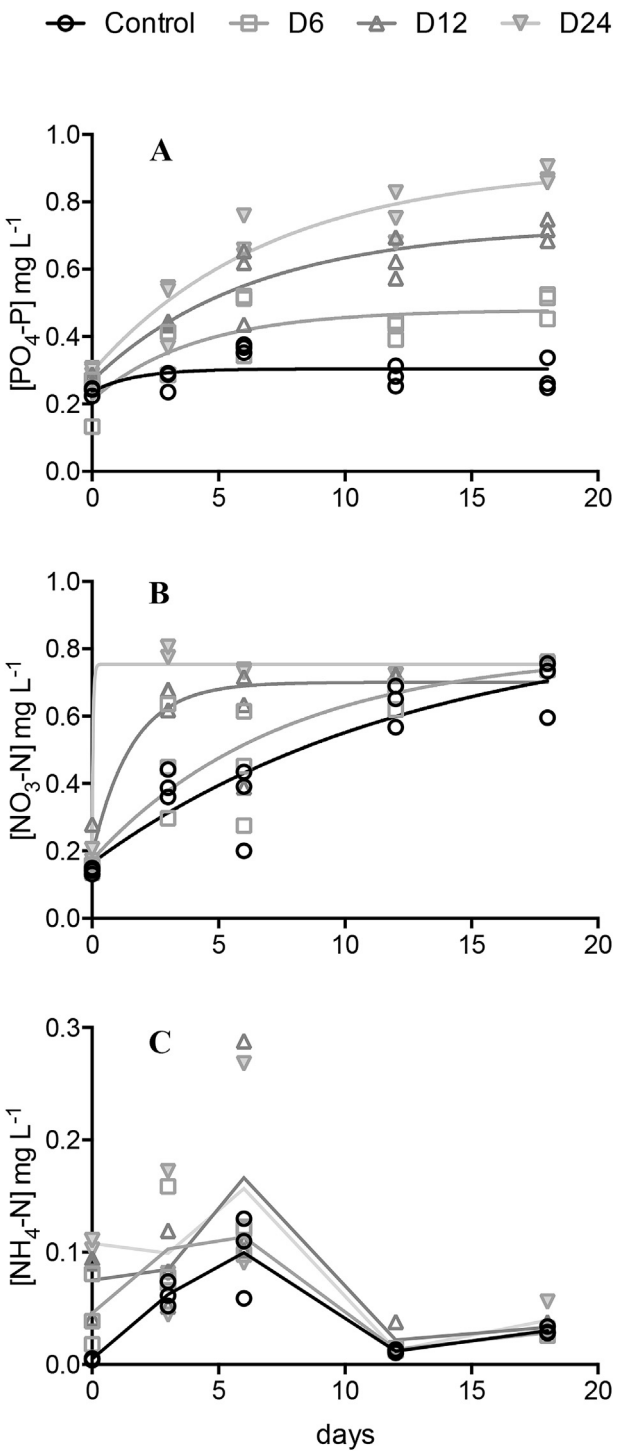

Fig. 3. The concentration of dissolved inorganic nutrients in the water column: over time for each density class. A fitted regression line was added to plot to clarify the variation patterns. A) PO_4-P; B) NO_3-N; and C) NH_4-N.

Hakenkamp, 2001), which may partially explain the higher phosphorus release in medium and small sized *C. fluminea*' treatments. Regarding density, phosphate release was favoured by increasing population density, suggesting that metabolic P release may also play a significant role in the phosphorus flux into the water column, as observed by Lauritsen and Mozley (1989).

Regarding the dynamics of DIN (NH_4-N + NO_3-N + NO_2-N), the presence of *C. fluminea* originated a net increase of DIN release into the water column, especially for medium and small-size treatments. Overall, while at the end of the experiment nitrate was the dominant form, accounting for circa 90% of DIN, in the first days of the experiment ammonium played an important role in the nitrogen cycle. The effect of bivalve presence in the nitrogen release probably resulted from a combination of mechanisms, similarly to what was observed for PO_4-P. Several reports accredited the increase of dissolved ammonium concentrations to both bivalve metabolism/excretion and bioturbation-mediated release from the sediment (Liu et al., 2007; Zhang et al., 2011). Bivalves excrete hypo-osmotic urine, consisting primarily of ammonia, with a variable rate depending on the species, individual size, temperature, stage in reproductive cycle and food availability (Vaughn and Hakenkamp, 2001 and references therein), with consequent increases in the water column ammonium concentration (Mortimer et al., 1999). In parallel, the syphoning activity of *C. fluminea* enhances solute exchange between overlying water and pore water, and thus helps ammonium diffusion from the sediment, while burrowing activities increased mineralization and decomposition rates of organic matter in sediments and ultimately enhanced ammonium efflux (Liu et al., 2007). Ammonium release was favoured in medium and small sized *C. fluminea*' tanks, highlighting the role of metabolic excretion in its dynamics, considering that the mass-specific nutrient excretion rates of animals (i.e. nutrients excreted per unit body mass per unit time) usually declines with increasing body mass (Vanni, 2002). The nitrate dynamics, on the other hand, may result from bioturbation-mediated sediment efflux, and also from the onset of bacterial nitrification (Liu et al., 2007), following the increase of dissolved ammonium in the first days of the experiment. While the presence of *C. fluminea* influenced nitrate release, the effect of bivalve size and density was less pronounced, possibly due to the minor dependence on bivalve metabolism. In fact, bivalve density did not affect the final dissolved NO_3-N concentrations, but rather the kinetics of its release from the sediments, favoured by the more intense bioturbation in higher density treatments.

These results highlight the importance of population structure on the dissolved nutrient dynamics, especially considering the die-off events reported for this species (Sousa et al., 2012; Oliveira et al., 2015). Its ability to rapidly repopulate previously colonized habitats following massive population crashes caused by extreme physical conditions will result in dense populations of *C. fluminea* composed mostly of juveniles (Franco et al., 2012; Sousa et al., 2008b), which will result in enhanced nutrient release into the water column when compared with more mature, structured populations.

4.2. Climate change scenarios

One of the main objectives of the experiment was to evaluate the effect of hypothetical climate change scenarios on the dissolved inorganic nutrient dynamics of *C. fluminea* colonized ecosystems, considering the predictions of the IPCC (2014). Overall, our results suggest that both drought (salinity increase) and heat wave (temperature increase) phenomena may have a significant impact on the nutrient dynamics in aquatic systems colonized by this alien invasive species. Specifically, the drought simulation (salinity increase) revealed an increase of NH_4-N release and reduced phosphate release. Ammonium efflux has been found to be salinity dependent, and to increase parallel to overlying water salinity (Giblin et al., 2010), through the diffusion of salts into and ammonium out of porewater. Weston et al. (2010) found that small increases in salinity in upper estuary sediments (from 0 to 8) may reduce the exchangeable pool of the total NH_4-N and increase the fraction of dissolved NH_4-N relative to adsorbed NH_4-N. The presence of *C. fluminea* seems to have amplified this diffusion process,

Table 3
Estimated kinetic parameters of the nutrient (PO_4-P and NO_3-N) release from the density experiment tanks (n = 3 for each time point). C0, concentration at time 0; Css, concentration at steady state; km: mineralization rate constant (d^{-1}); $T_{b1/2}$: half-life (d); R_2: determination coefficient; SE: standard error.

		C_0 (mg L^{-1}) ± SE	C_{SS} (mg L^{-1}) ± SE	K_m (d^{-1}) ± SE	$T_{b1/2}$	R^2	$S_{x/y}$
PO_4-P	Control	0.24 ± 0.03	0.30 ± 0.02	0.59 ± 0.90	1.18	0.29	0.046
	D6	0.21 ± 0.04	0.48 ± 0.04	0.25 ± 0.04	2.81	0.70	0.071
	D12	0.27 ± 0.03	0.73 ± 0.05	0.16 ± 0.05	4.37	0.90	0.059
	D24	0.29 ± 0.04	0.91 ± 0.07	0.14 ± 0.07	4.90	0.92	0.067
NO_3-N	Control	0.16 ± 0.05	0.88 ± 0.26	0.077 ± 0.053	8.97	0.84	0.095
	D6	0.17 ± 0.06	0.79 ± 0.12	0.13 ± 0.07	5.18	0.80	0.11
	D12	0.19 ± 0.06	0.70 ± 0.04	0.64 ± 0.31	1.08	0.83	0.010
	D24	–	–	–	–	–	–

probably as a result of bioturbation, and can therefore have a significant impact in DIN release from the sediment to the water column. This increase of ammonium efflux was not reflected on dissolved nitrate concentrations, even after the drop in NH_4-N concentrations (between day 6 and 12 of the experiment), which is in agreement with a previous study (Turek and Hoellein, 2015). The authors observed that *C. fluminea* significantly increased NH_4-N and N_2 flux out of sediments but did not affect NO_3-N flux, suggesting the presence of coupled nitrification–denitrification processes (Turek and Hoellein, 2015).

Contrary to the most common behaviour in transitional ecosystems with salinity gradients, the increase in salinity did not favor phosphate release into the water column; instead, dissolved PO_4-P concentrations decreased in all treatments except in the medium sized *C. fluminea*. P–Fe–S interactions may promote the release of PO_4-P from the sediments with increasing salinity, probably due to the dissolution of iron (hydr)oxides, the formation of iron (II) sulfides and the release of iron-bound P (Coelho et al., 2004; Jensen et al., 1995). Given that phosphate desorption from ferric iron (hydr)oxides is enhanced with increasing pH (Spiteri et al., 2008), the stability observed in pH values throughout the different treatments and over time in the present experiment may partially explain the phosphate behaviour pattern. Additionally, the possible formation of ternary Mg-phosphate surface complexes may have prevented, or at least limited the extent of phosphate desorption with increasing salinity (Spiteri et al., 2008).

The heat wave scenario (temperature increase), in turn, only enhanced NH_4-N release to the water column, in synergy with the salinity effect, while coupled to the depletion of dissolved oxygen concentrations. A similar pattern of increased NH_4-N effluxes with temperature in the presence of *C. fluminea* were reported previously (Zhang et al., 2013), coupled with higher sediment oxygen uptake and PO_4-P release. This behaviour may result from the reductive dissolution of easily reducible Fe oxides due to the depletion of oxygen in the top sediment layers from bivalve respiration (Chen et al., 2016). However, those results reflect a 10 °C change in temperature, from 15 to 25 °C, while the measured temperature differential in the present study was lower (6 °C) and in the upper physiological range of the species (from 24 to 30 °C). In a different study, Xiao et al. (2014) found similar oxygen consumption rates and NH_4-N excretion rates for *C. fluminea* at 25 °C and 32 °C, which may suggest that changes within this temperature range have little effect on the metabolism of this bivalve, and that the increase of NH_4-N efflux may have a source other than metabolic, such as bioturbation and bioirrigation. Bivalve activity will enhance nutrient regeneration and enhanced diffusion through the burrow walls, especially with higher water temperatures, which will promote higher NH_4-N efflux from the sediments (Kemp et al., 1990). If this is the case, the oxygen depletion in 30 °C tanks will not derive from bivalve metabolism, and may have, in turn, constrained the species response during the experiment, due to its sensitivity to low oxygen concentrations.

Overall, the present research highlights the significant effect of *C. fluminea* in ecosystem nutrient release and dynamics, enhancing the efflux of both phosphate and DIN into the water column. Concerning the climate change scenarios tested, both drought (salinity increase) and heat wave (temperature increase) events will have an impact on the DIN dynamics within *C. fluminea* colonized systems, favouring a higher NH_4-N efflux, especially in the first days after a shift in environmental conditions. The population structure of this IAS on any given system will have a decisive role on the impact of the species, as a predominantly juvenile population was found to promote stronger nutrient fluxes into the water column.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ecss.2018.03.001.

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